Association of Fibril Structure Formation with Cell Surface Properties of *Yersinia enterocolitica*

Adherence of various pathogenic bacteria to eucaryotic cells has been demonstrated to play a central role in their ability to colonize epithelial surfaces (24). Both specific and nonspecific interactions have been shown to mediate the adherence of certain pathogens. Specific interaction involves the binding of a specific surface structure on the bacterial cell surface with receptors on the epithelial cells. Nonspecific interaction depends on physicochemical forces between the surfaces of the epithelial tissue and bacterial cells. Formation of fimbriae has been associated with cell surface hydrophobicity among various pathogens (7, 10).

There is considerable interest in the pathogenicity of *Yersinia enterocolitica* since a resident plasmid contributes to its virulence (9, 21–23, 27). Although various properties of *Y. enterocolitica* are associated with the virulence plasmid (9, 21–23, 28), attachment to the mucosal surface is thought to be chromosomally mediated (12, 17, 23). Certain strains have been reported to elaborate fimbriae (19) which mediate increased cell surface hydrophobicity (6). However, low growth temperatures (<30°C) are required for their synthesis. Moreover, no correlation has been observed between the formation of fimbriae and the attachment to epithelial cells in vitro (19). We have recently reported the association of mouse gastrointestinal tract colonization with the plasmid-mediated cell surface properties of *Y. enterocolitica* (13, 14). In the present study, the formation of a fibrillar material associated with the virulence plasmid and the cell surface properties of *Y. enterocolitica* are described.

We investigated in greater detail the autoagglutination (AA) reaction, which is one of the properties associated with the virulence of *Y. enterocolitica* (15, 22, 28). Plasmid-bearing *Y. enterocolitica* MCH56A2 (p^+; serotype O:3) was AA^+ at 37°C but not at 22°C; its plasmidless derivative (p^-) was AA^-, regardless of the growth temperature, as described in a previous report (14). During growth at 37°C in Eagle minimal essential medium plus 10% fetal calf serum, p^+ cell aggregates adhered to the wall of the polystyrene test tube and eventually sank to the bottom of the tube. Some remained attached to the tube wall; these were best observed by staining with crystal violet. The AA reaction of p^+ cells is in marked contrast to the autoaggregation of fimbriated bacteria which form thick pellicles at the broth-air interphases of undisturbed still cultures (6, 20).

Under phase-contrast light microscopy, the p^+ cells grown at 37°C were shown (Fig. 1) to form long chains (some chains consisted of 12 cells) which were attached to each other and to the glass surface. None of these were observed when p^+ cells were grown in brain heart infusion or tryptic soy broth (Difco Laboratories, Detroit, Mich.). The p^- cells grew at 37°C as single, unattached pleomorphic cells. At 22°C, both strains were motile, short rods.

Transmission electron microscopy was employed to determine the formation of the surface structure on p^+ cells. From our previous study (13), p^+ cells grown at 37°C were shown to exhibit a highly anionic surface. This information suggested to us the efficacy of ruthenium red (RR; Sigma Chemical Co., St. Louis, Mo.) for staining the putative surface structure. This electron-dense cationic stain has been useful in visualizing anionic polysaccharide fibers (glycocalyx) on the surface of various bacteria (5). Pellets of cells or mixtures of cells and latex particles (0.5 μm in diameter; E. F. Fullum, Inc., Schenectady, N.Y.) were fixed in 1.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) containing 0.05% RR at 22°C for 2 h. After a brief rinse in cacodylate buffer, the pellets were postfixed in 2% OsO_4 in cacodylate buffer containing RR for 1 h at 0°C. After washing the pellets with cacodylate buffer containing RR, they were dehydrated with graded series of ethanol and embedded with Epon 812 by the method of Luft (16). Sections were cut and examined with a Philips model 200 electron microscope at 60 kV.

The plasmid-bearing cells grown at 37°C elaborated a matrix of fibers distal to the outer membrane (Fig. 2A). This fibrillar material appeared to facilitate the attachment of the cells to each other. Recently, we reported this cell-cell interaction (AA) to be accelerated by the addition of divalent cations such as Ca^{2+} or Zn^{2+}, which are thought to form a bridge between the anionic surfaces of the cells (14). The cells also interacted with the hydrophobic latex particles. Interestingly, the latter were coated with what appeared to be fibrillar material that may have been dislodged from the cell surface. No fibrillar component was observed on plasmid-bearing cells previously grown at 22°C (Fig. 2B) or on plasmidless cells, regardless of the growth temperature.

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FIG. 1. Phase-contrast micrograph showing chain formation and clumping of *Y. enterocolitica* MCH56A2 (p^+) cells grown in Eagle minimal essential medium plus 10% fetal calf serum. Magnification, ×1,500.

The fact that the fibrous matrix was demonstrated despite the absence of any stabilizing influence suggests that the matrix is not as highly hydrated as the extracellular polysaccharide matrix exhibited by other bacteria (5). Yet, it is possible that a better image of the surface matrix could be attained with the use of stabilizing influences such as lectins or antiserum (5) or the employment of different dehydration techniques (25). The flexible nature of the fibril structure is similar to the capsular antigen of *Yersinia pestis* described by Chen et al. (4). The flexible fibrillar material on the cell surface of *Y. enterocolitica* and the rigid gonococcal and *Escherichia coli* type 1 fimbriae share the property of attachment to latex particles (2, 3), indicating their hydrophobic nature.

Our results suggest that the fibrillar material of *Y. enterocolitica* mediates the surface properties of the cell which remained undiminished after treatment with sodium metaperiodate (10 mM, 30 min) and actually increased when heated at 60°C for 60 min (data not shown). These observations indicate that the fibrillar material is flexible, amphipathic protein similar to β-casein (18). It is possible that some of the plasmid-mediated outer membrane proteins elaborated by *Y. enterocolitica* (1, 17, 23) may comprise components of the fibril structure.

Our results indicate that the AA reaction is based on the formation of long chains of cells and the alteration of cell surface properties likely to be mediated by the fibrillar material. The recently described mannose-resistant aggluti-

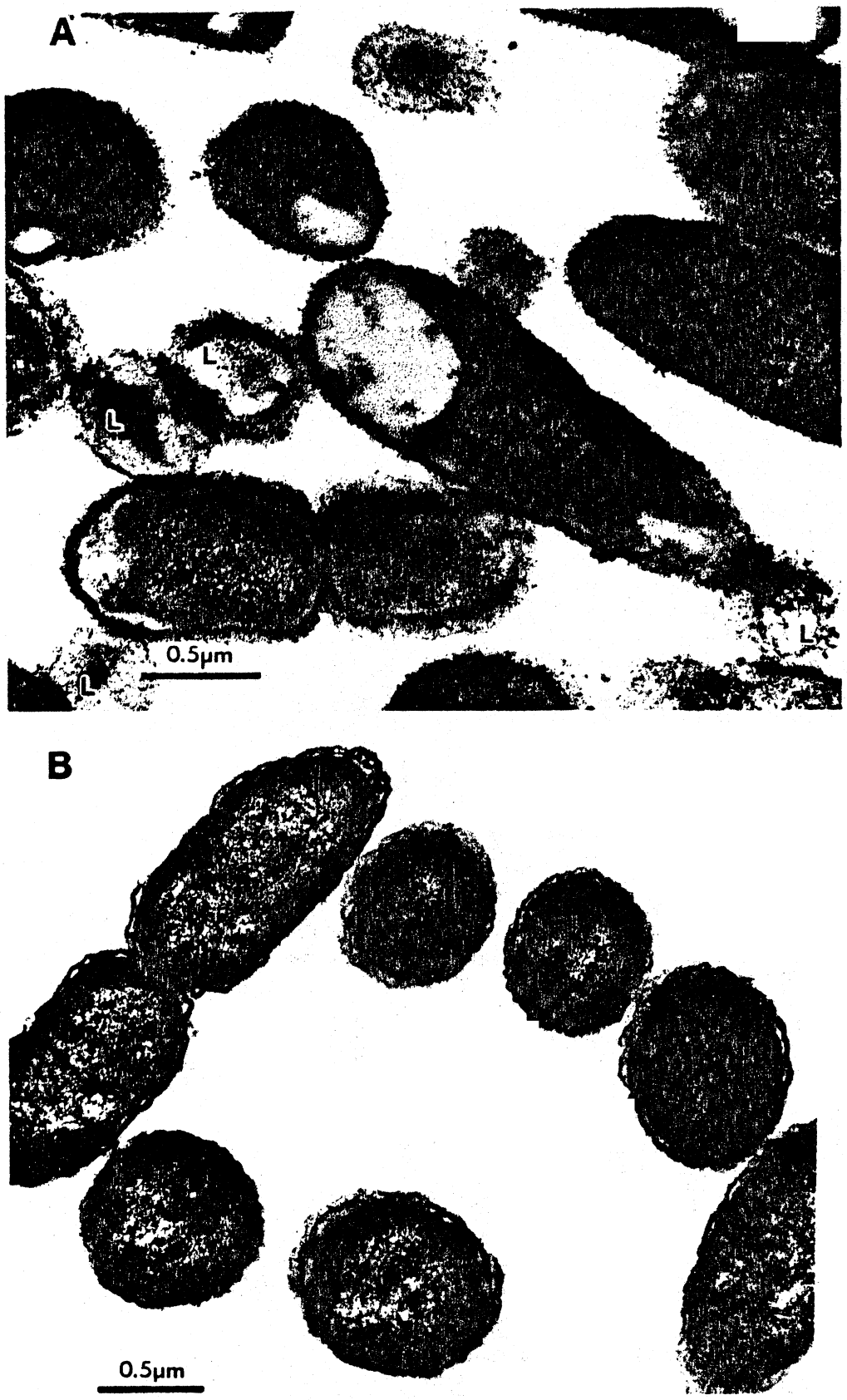

FIG. 2. Electron micrographs of RR-stained p^+ cells grown at 37°C (A) and 22°C (B). Note the presence of fibrous material on the surface of the cells grown at 37°C and on latex particles (L) but not on cells grown at 22°C.

nation of guinea pig erythrocytes by virulent cells of *Y. enterocolitica* (11) is also likely to be mediated by the fibril structure. It is envisaged that this surface structure promotes the attachment of p^+ cells to the intestinal mucosa. The increased susceptibility to phagocytosis as a result of the increased surface hydrophobicity (26) may be counteracted by the formation of long chains and microcolonies (5). The fibril structure may act also as a molecular barrier against the killing action of serum (21).

It should be emphasized that the surface structure described in this report is distinct from the rigid appendages (fimbriae) which are elaborated by certain yersiniae after several subculturings at low (<35°C) but not high (>35°C) temperatures (6, 19). Presumably, the expression of the fimbriae is chromosomally encoded among the environmental group of yersiniae (11). Whether virulent strains of *Y. enterocolitica* are capable of elaborating fimbriae is not known.

Studies are in progress as to the determination of whether the fibril structure or the plasmid-mediated outer membrane proteins or both play the major role in mediating the altered cell surface properties of virulent strains of *Y. enterocolitica*.

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